5. BIOPOLYMER ORGANIZATION OF THE EXINE OF JUNIPERUS VIRGINIANA L., AND TAXUS BACCATA L.

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Abstract

Pollen grains of *Juniperus virginiana* L. and *Taxus baccata* L. were partially degraded with five different combination of solvents and oxidizing agents. These experiments were used following two basic methods: 1. Without heating before the solvent and oxidizing procedure. 2. The pollen grains were heated at 100 °C for one hour before the partial degradation. The solvated exines were fragmented with a magnetic stirrer in watered medium, for 30 minutes. With the transmission electron microscope several kinds of organization levels were observed, such as basic regular pentagonal polygons, PENROSE-like units, and their highly organized structures.

Key words: Palynology, *Gymnospermae*, inaperturate types, biopolymer structure.

Introduction

In our previous paper (KEDVES and ROJIK, 1989) partially degraded pollen grains of *Alnus glutinosa* (L.) GAERTN. were fragmented before the TEM studies. The advantages and the disadvantages of this combined method are pointed out in this paper. The fragmentation method of the partially degraded sclereids of *Armeniaca vulgaris* LAM. (KEDVES and ROJIK, 1991), and at the colonies of *Botryococcus braunii* KÜTZ, from the oil shale (KEDVES et al., 1991) resulted in surprising and interesting biopolymer structures. The finding of the basic pentagonal polygon biopolymer units and their highly organized PENROSE-I like structures in the sclereids of the endocarpium of *Armeniaca vulgaris* LAM. yields another opportunity to continue experiments for a new energy basis based on the energy which may be liberated during the disintegration of the biologic quasi-crystalloid biopolymer structures. Sclereids, and the *Botryococcus* colonies of the oil shale seem to be the most suitable for this purpose. But it is necessary to call attention to researches of the explosive dangerous coal pulver, and in general to the coal investigations, too. Two important purposes may be pointed out here:

I. The elimination or the diminution of the danger of explosion of the coal pulver in mines.

II. To try to utilize the energy of the explosion of the coal pulver.

In this paper, the results of two inaperturate gymnosperm pollen grains are

summarized. The purpose of these investigations is to get a progress in the knowledge of the biopolymer structure of the gymnosperm pollen grains.

Materials and Methods

Juniperus virginiana L.

Collected: Dr. I. KINCSEK in the Botanical Garden of J. A. University on 10. 3. 1989.
Experiment numbers: 503-512.

Taxus baccata L.

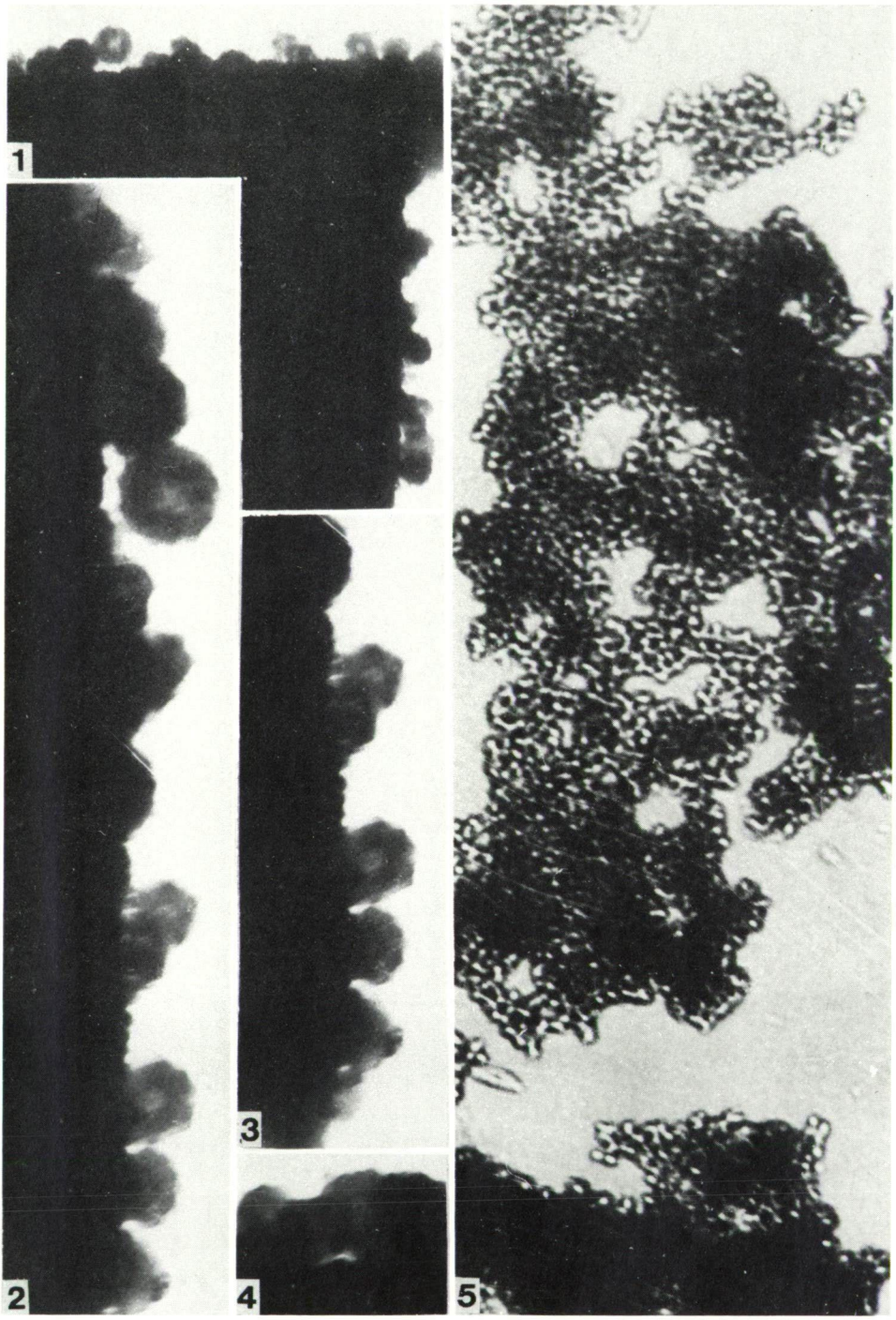
Collected: A. BARTOS in the Garden of the University building on 13. 3. 1989.
Experiment numbers: 513-521.

EXPERIMENTS

The experiments were made between 16. 3. 1989. – 21. 3. 1989.

Experiment numbers		
without heating	heated on °C 100 during 1^h	
503, 513	508, 518	20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h
504, 514	509, 519	20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h, washing (H_2O) + 10 ml 1% $KMnO_4$ aq. dil., temperature 30 °C, lenght of time 24^h
505, 515	510, 520	20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h, washing (H_2O) + 10 ml $KMnO_4$ aq. dil., temperature 30 °C, length of time 48^h
506, 516	511, 521	20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h, washing (H_2O) + 10 ml 1% $KMnO_4$ aq. dil., temperature 30 °C, length of time 24^h, washing (H_2O) + 2 ml acetic acid anhydride, temperature 30 °C, length of time 24^h
507, 517	512, 522	20 mg air dried pollen grains + 1 ml 2-aminoethanol, temperature 30 °C, length of time 24^h, washing (H_2O) + 10 ml 1% $KMnO_4$ aq. dil., temperature 30 °C, length of time 24^h, washing (H_2O) + 5 ml methanol, temperature 30 °C, length of time 24^h

The following process is identical with those published in our previous paper (1989), p. 72: "After the partial degradation of the pollen grains the residues were washed in distilled water. The fragmentation was made with a magnetic stirrer in watered medium, during 30 minutes. The fragmented exines were dropped on a grid covered with collodium pellicle and then dried. The electron microscopical investigations were made on a Tesla BS-500 transmission electron microscope, resolution 6 Å."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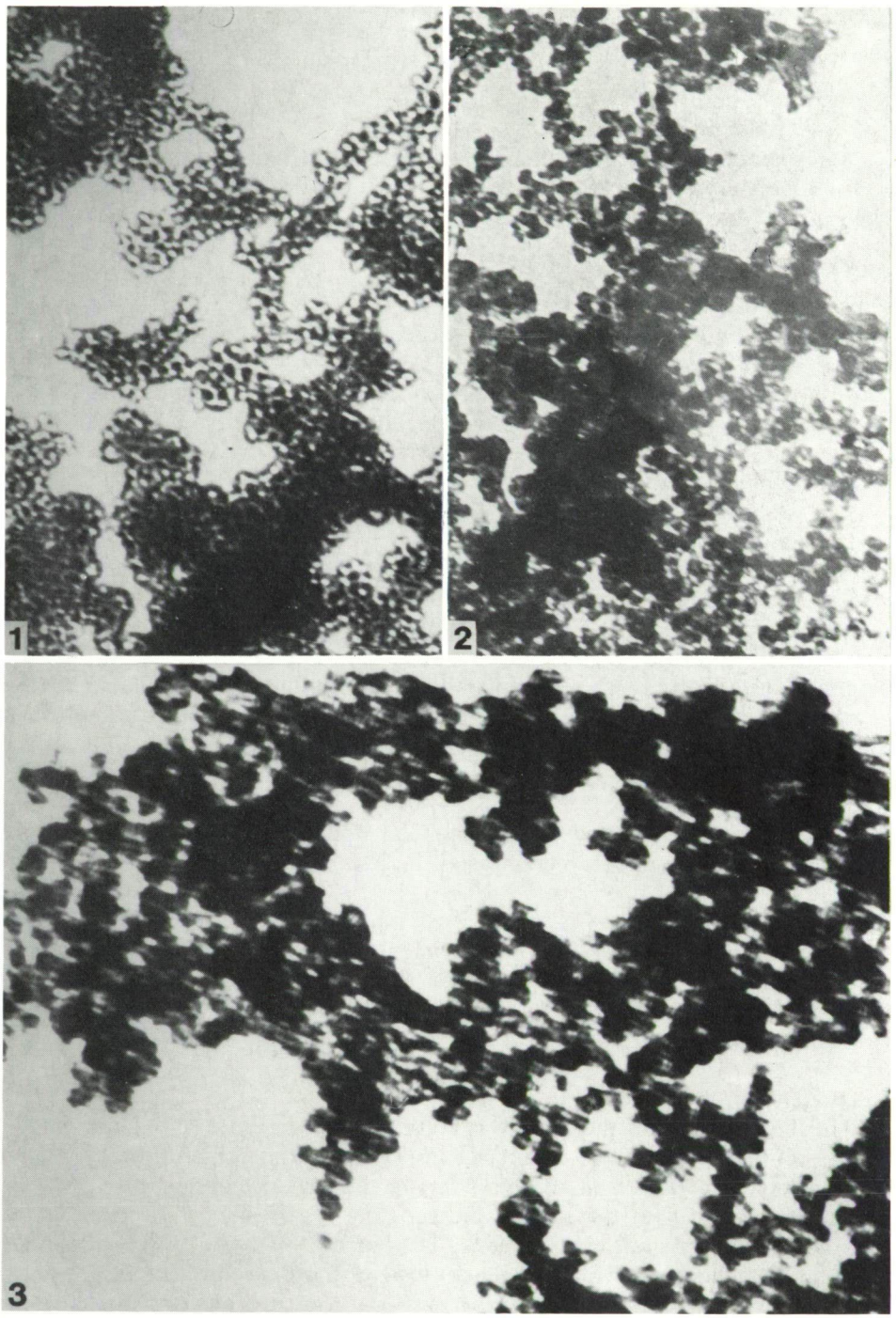
◀ Plate 5.1.

1—5. *Juniperus virginiana L.*

1—4. Experiment No: 503, outer surface of the pollen grain, covered with orbiculi.
1. Negative no: 9171, 20.000x.
2—4. Negative no: 9171, 50.000x.
5. Experiment No: 504, characteristic biopolymer structure of different organization levels. Negative no: 9182, 250.000x.

Plate 5.2. ▶

1—3. *Juniperus virginiana L.*
1,2. Experiment No: 505, two kinds of biopolymer structure preservation. Negative no: 9186, 250.000x.
3. Experiment No: 506, biopolymer structure of the pollen wall. Negative no: 9296, 100.000x.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Results

Juniperus virginiana L.

Experiment No: 503 (Plate 5.1., figs. 1—4)

This experiment has not sufficiently degraded the exine to the investigation of the biopolymer organization of the exine. The orbiculi on the surface of the pollen grain are illustrated. Inside the orbiculi no molecular structure was observed.

Experiment No: 504 (Plate 5.1., fig. 5)

Well defined biopolymer structures were observed. These molecular systems are of different organization levels. Light polygons of 8—16 Å in diameter with central globular elements of strong electron density are the basic regular pentagonal polygon units. The diameter of the highly organized PENROSE-I like, nearly globular units is 26—38 Å. These units are arranged into filaments and/or larger mostly irregular polygon systems.

Experiment No: 505 (Plate 5.2., fig. 1,2)

Regarding the fine organization and the electron density of the particles, two kinds of biopolymer structures were observed. One is similar to the previously discussed one (Plate 5.2., fig. 1). But the basic biopolymer units are mostly arranged into filaments. The filamental units are arranged into irregular larger polygons. The second type of biopolymer structure illustrated in Plate 5.2., fig. 2., is composed of electron dense globular units which may occur of different kinds of high organization.

Experiment No: 506 (Plate 5.2., fig. 3)

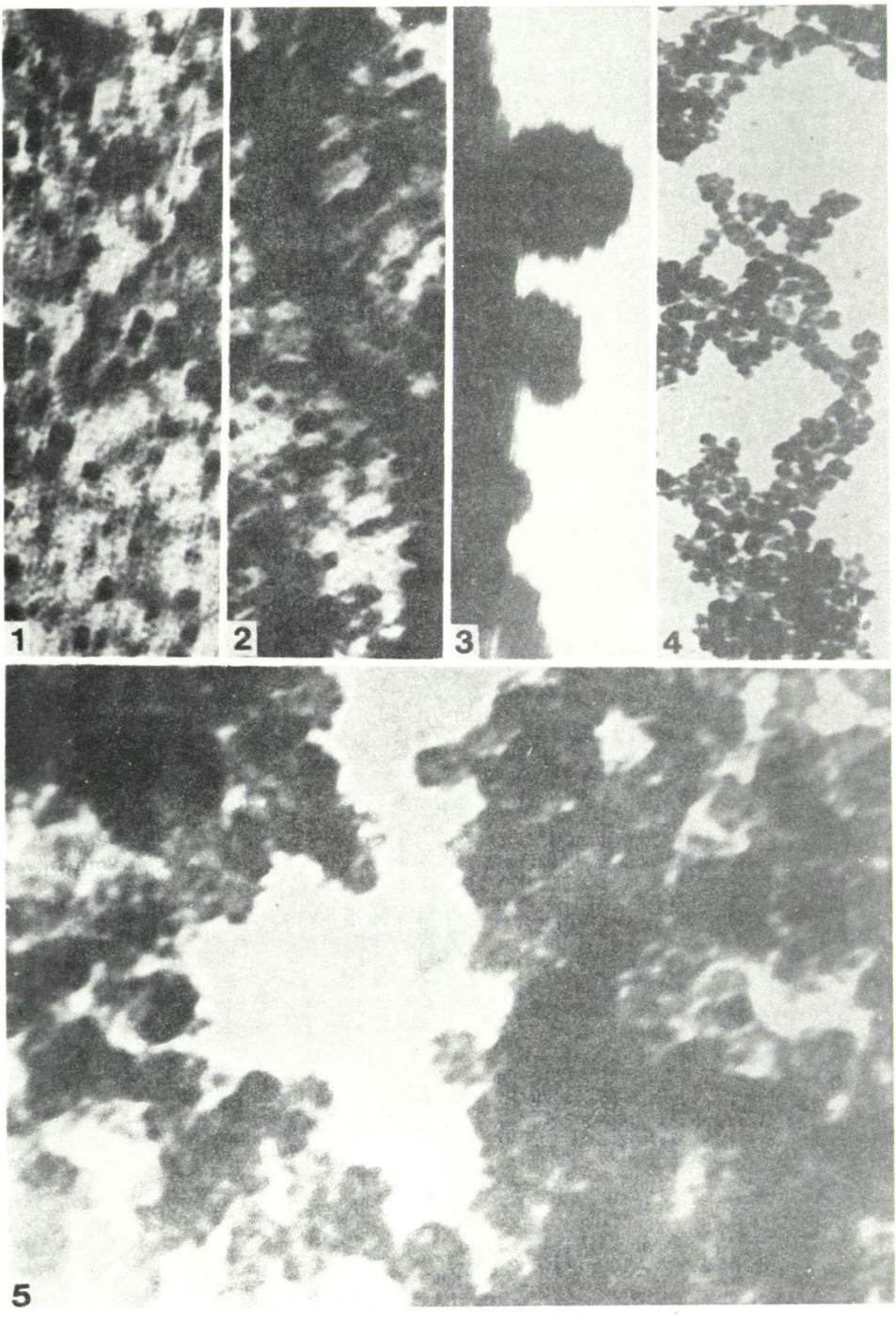
This experiment resulted in dark globular units of 15—30 Å. The arrangement of these units is not so regular as at the previous experiment.

Experiment No: 507 (Plate 5.3., fig. 1,2)

In consequence of this kind of experiment light — negative — regular basic pentagonal units were observed together with the electron dense network of pentagon biopolymer system. In general an advanced degradation may be established in the biopolymer system.

Experiment No: 508 (Plate 5.3., fig. 3,4)

The TEM picture of the surface is a little similar to those of experiment No 503. As difference the more compact consistence of the exine and the orbiculi can be pointed out. This is in all probability in consequence of heating before the partial degradation with the solvent method. Fig. 3. of Plate 5.3. illustrate the surface of the pollen grain with orbiculi. But together with the above mentioned fragments globular biopolymer structures were also observed (Plate 5.3., fig. 4). The diameter of these globular units is about 14—25 Å, the electron affinity is on a high level. The arrangment (linear and/or network-like) can be pointed out.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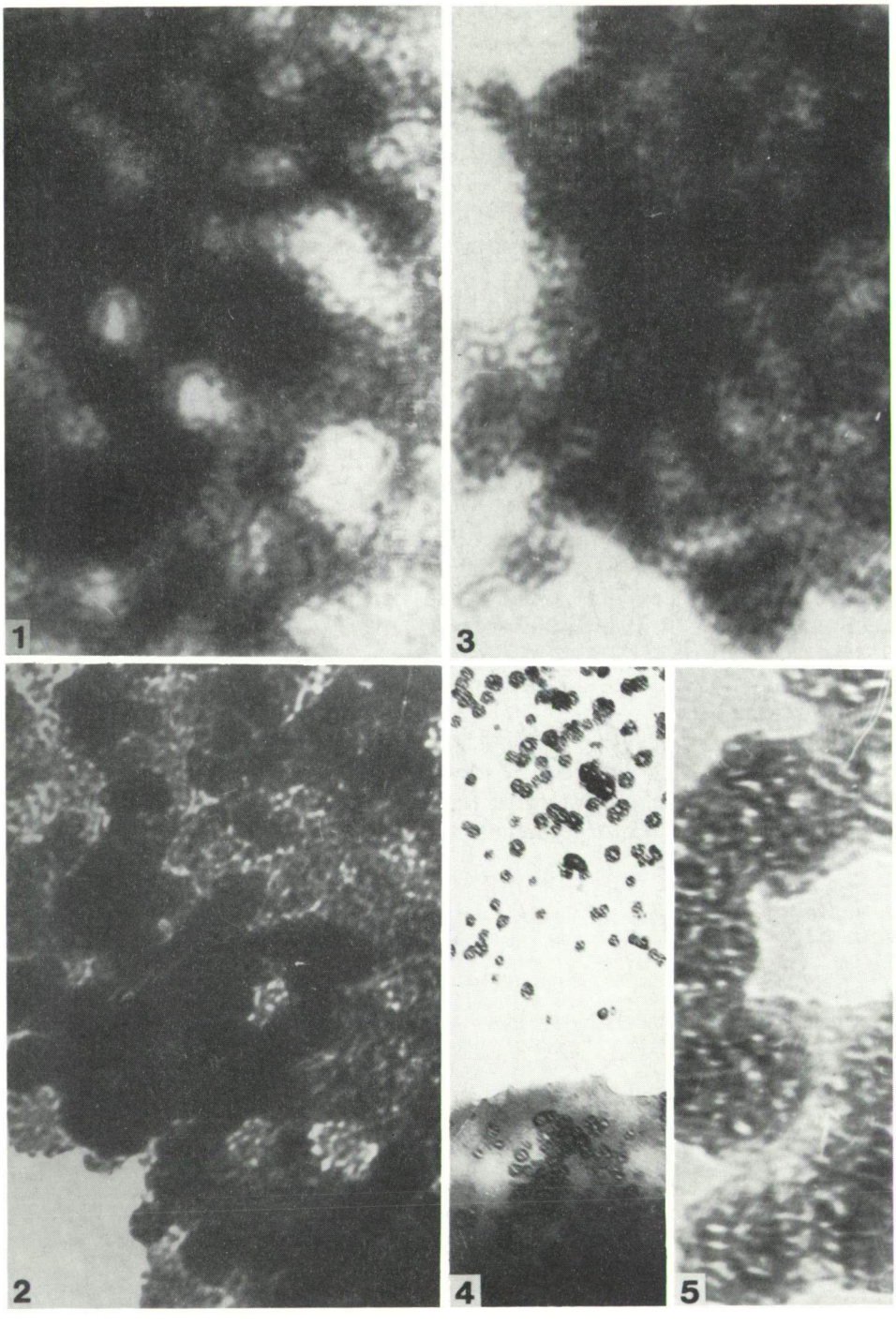
◀ Plate 5.3.

1—5. *Juniperus virginiana* L.
1,2. Experiment No: 507, biopolymer structure of the exine. The basic pentagonal polygon units are of two different characters. Negative no: 9305, 100.000x.
3,4. Experiment No: 508.
3. Outer surface of the pollen grain with orbiculi. Negative no: 9189, 48.000x.
4. Degraded biopolymer structure of the pollen wall. Negative no: 9191, 100.000x.
5. Experiment No: 509, biopolymer structure of the exine. Negative no: 0028, 250.000x.

Plate 5.4. ▶

1—5. *Juniperus virginiana* L.
1. Experiment No: 510, biopolymer structure of the pollen wall. Negative no: 9224, 250.000x.
2,3. Experiment No: 511, biopolymer organization of the exine.
2. Negative No: 9333, 150.000x.
3. Negative No: 9314, 150.000x.
4,5. Experiment No: 512.
4. The surface of the pollen grain with attached and dispersed orbiculi. Negative no: 9340, 25.000x.
5. Damaged biopolymer structure. Negative no: 9338, 250.000x.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Experiment No: 509 (Plate 5.3., fig. 5)

A combined globular biopolymer structure was observed. The large units are of 28—56 Å in diameter. This kind of biopolymer organization is extremely similar to those of the globular units of the oil shale organic material (*Botryococcus braunii* KÜTZ., KEDVES et al., 1991, p. 29). This is without doubt highly organized system in this way the PENROSE-I, or II biopolymer unit.

Experiment No: 510 (Plate 5.4., fig. 1)

Not so well defined basic pentagonal polygon units were observed. The highly organized globular structures are also not so characteristic, but the larger polygons are well illustrated in fig. 1, Plate 5.4.

Experiment No: 511 (Plate 5.4., fig. 2,3)

This experiment brought essentially the same result as the previously discussed one.

Experiment No: 512 (Plate 5.4., fig. 4,5)

The low magnified picture (Plate 5.4., fig. 4) well illustrates the orbiculi which are dispersed from the surface. The biopolymer structure of the exine is destroyed. Globular units of 60—100 Å in diameter were observed. These structures are damaged PENROSE-like biopolymer systems. The arrangement of these globular units is filamentous or large polygons.

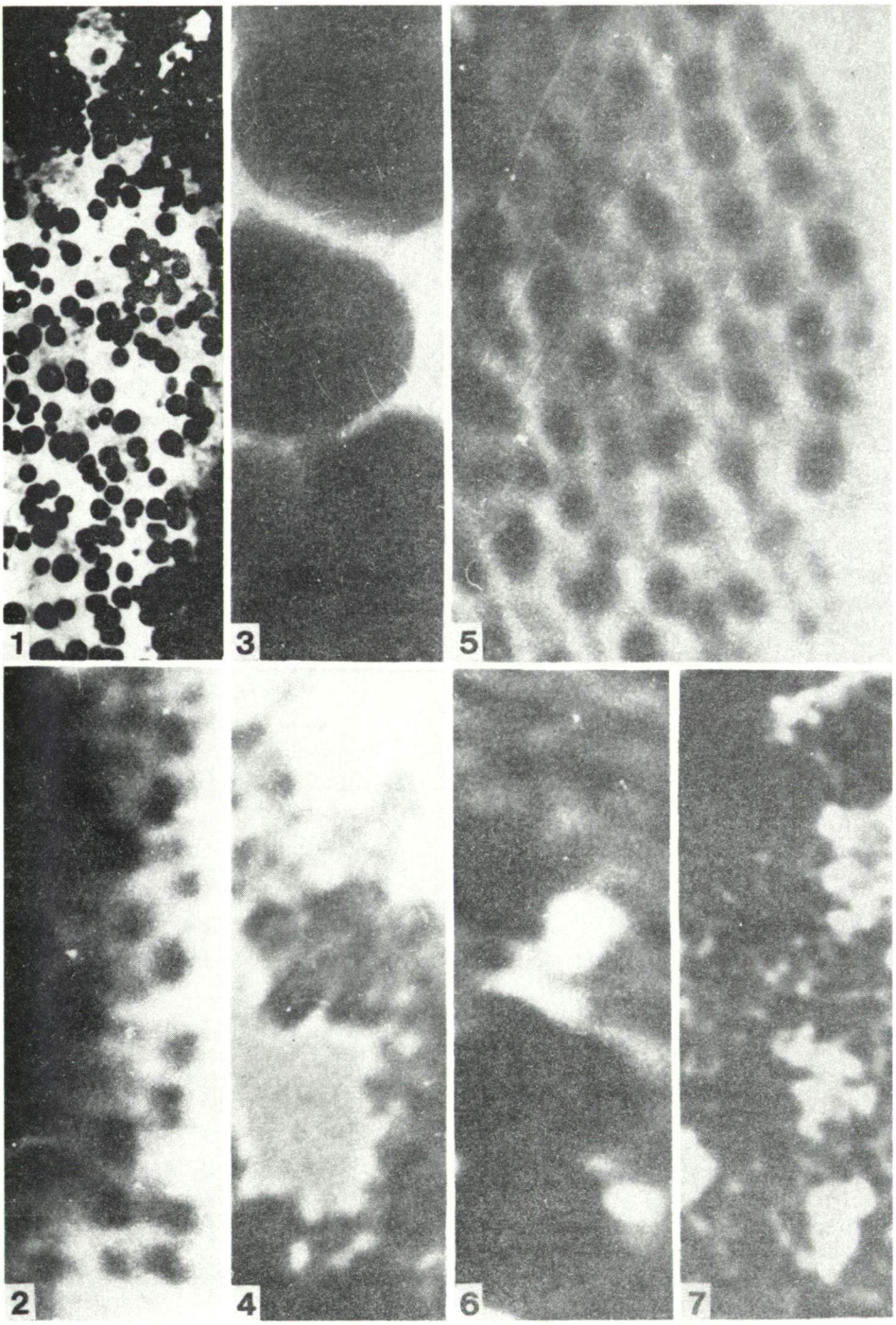
Taxus baccata L.

Experiment No: 513 (Plate 5.5., fig. 1,3)

The low magnified pictures well illustrate the surface of the pollen grain with attached orbiculi which are of extreme electron density. Biopolymer structures were not observed at this experiment.

Plate 5.5 ►

1—7. *Taxus baccata L.*
1,3. Experiment No: 513, the surface of the pollen grain with attached orbiculi.
1. Negative no: 9226, 10.000x.
3. Negative no: 9228, 100.000x.
2,5. Experiment No: 514, the surface of the pollen grain with attached orbiculi.
2. Negative no: 9249, 500.000x.
5. Negative no: 9250, 500.000x.
4. Experiment No: 515, damaged biopolymer structure of the pollen wall. Negative no: 9257, 500.000x.
6. Experiment No: 516, damaged biopolymer structure of the pollen wall. Negative no: 9350, 500.000x.
7. Experiment No: 517, biopolymer structure of the pollen wall. Negative no: 9252, 500.000x.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Experiment No: 514 (Plate 5.5., fig. 2,5)

This experiment resulted in different kinds of biopolymer remains. Among these the light-coloured polygon system with globular units in the centre of the "meshed" of each polygon. These "central" globular biopolymer units have strong electron density. The investigation of this "negative" polygon system with the modified MARKHAM rotation method may be the subject of further investigations.

Experiment No: 515 (Plate 5.5., fig. 4)

Not so clearly characteristic biopolymer units were observed. The diameter of these structures are of 24—40 Å approximatively.

Experiment No: 516 (Plate 5.5., fig. 6)

The results of this experiment are similar or nearly identical to the previously mentioned and discussed one.

Experiment No: 517 (Plate 5.5., fig. 7)

Globular units of strong electron affinity were observed. The diameter of these units is 6—8 Å, and forms a quasi-crystalloid lattice. Not so characteristic PENROSE-like globular units were also observed.

Experiment No: 518 (Plate 5.6., fig. 1)

The surface of the pollen grains is seemingly damaged. The orbiculi on the surface are of strong electron density, and its surface coni (spinules) are also degraded.

Experiment No: 519 (Plate 5.6., fig. 3)

Globular units of strong electron density were observed forming more or less pentagonal polygons. Not so characteristic highly organized globular units and their linear and irregular network occurred also during our investigations.

Experiment No: 520 (Plate 5.6., fig. 4)

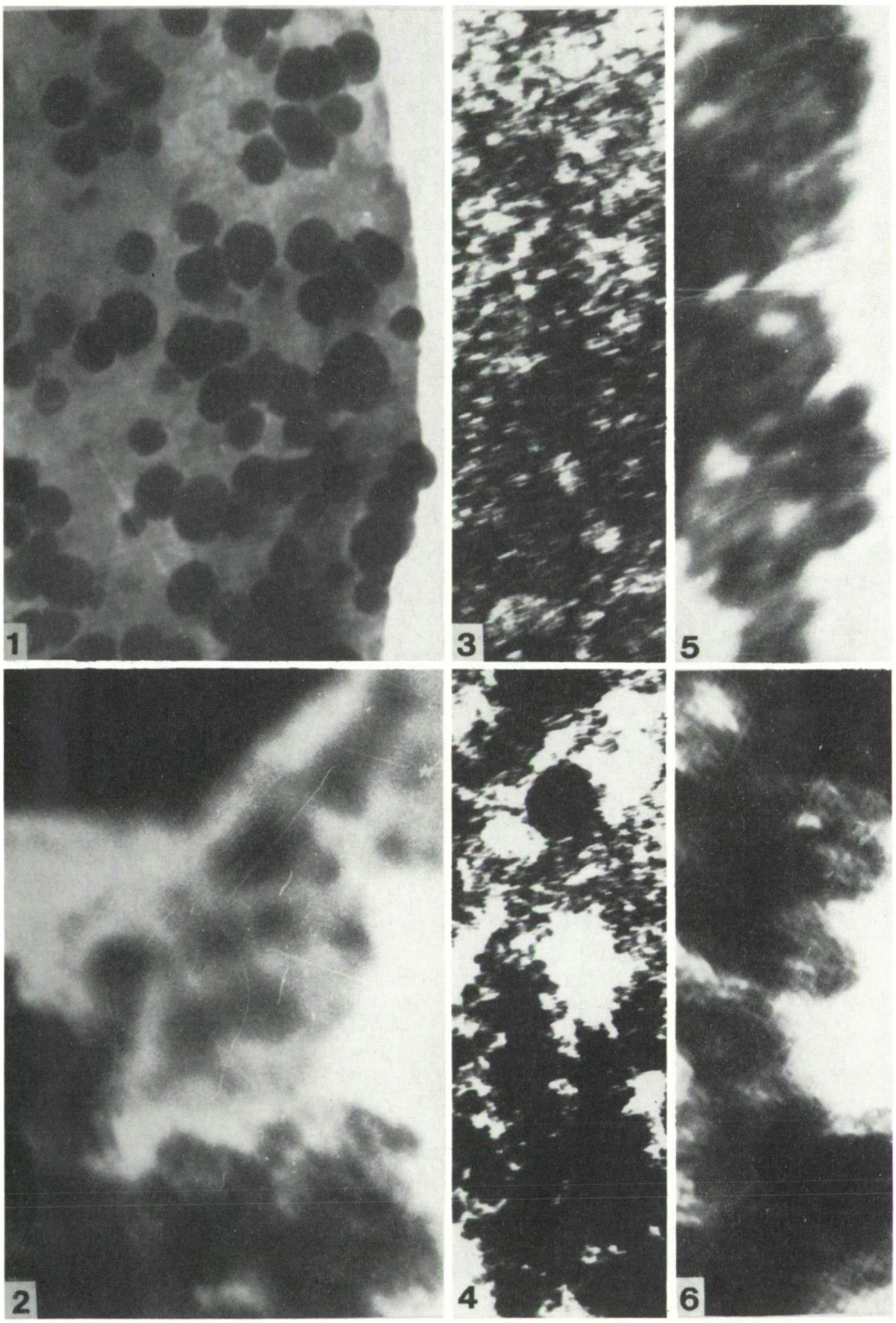
The results of this experiment are similar to the previously discussed one.

Plate 5.6. ▶

1—6. *Taxus baccata* L.

1. Experiment No: 518, surface of the pollen grain with attached orbiculi. Negative no: 9282, 25.000x.

2. Experiment No: 521, biopolymer structure of the pollen wall. Negative no: 9358, 500.000x.
3. Experiment No: 519, biopolymer structure of the pollen wall. Negative no: 9286, 50.000x.
4. Experiment No: 520, biopolymer structure of the pollen exine. Negative no: 9295, 50.000x.
5. Experiment No: 521, biopolymer structure of the pollen wall. Negative no: 9359, 500.000x.
6. Experiment No: 522, biopolymer structure of the pollen wall. Negative no: 9365, 250.000x.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Experiment No: 521 (Plate 5.6., fig. 2,5, plate 5.7.)

The low magnified picture of the exploded pollen grain is illustrated in Plate 5.7. The biopolymer structures of the outer surface and of the orbiculi are well shown. In the highly magnified pictures damaged units of 60—90 Å in diameter were observed. Inside these units there are smaller ones with strong electron density. The diameter is about 8—12 Å. This biopolymer structure is also similar to those observed on the colonies of the *Botryococcus* algue extracted from the oil shale from Pula (Transdanubia, Hungary).

Experiment No: 522 (Plate 5.6., fig. 6)

Relatively well preserved basic biopolymer units and their highly organized structures were observed, including the PENROSE-I like organization.

Discussion and Conclusions

These new data demonstrated the advantages of this method. The TEM study of the fragments brought very useful complementary data to those of the ultrathin sections. The necessity to use combined methods to solve one problem can be emphasized in this place also.

The highly organized biopolymer structures of the exines presented in this paper are similar to or more or less identical with the previously described ones from the wall of *Botryococcus braunii* KÜTZ. (KEDVES et al., 1991) and *Alnus glutinosa* (L.) GAERTN. (KEDVES and ROJIK, 1989).

Acknowledgements

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Plate 5.7. ▶

Taxus baccata L.

Experiment No: 521, pollen grain after explosion. The biopolymer structure is well shown. Negative no: 9360, 60.000x.